MYCOLOGIA

VOL. I JULY, 1909 NO. 4

STUDIES IN PYROPHILOUS FUNGI—I. THE OCCURRENCE AND CULTIVATION OF PYRONEMA

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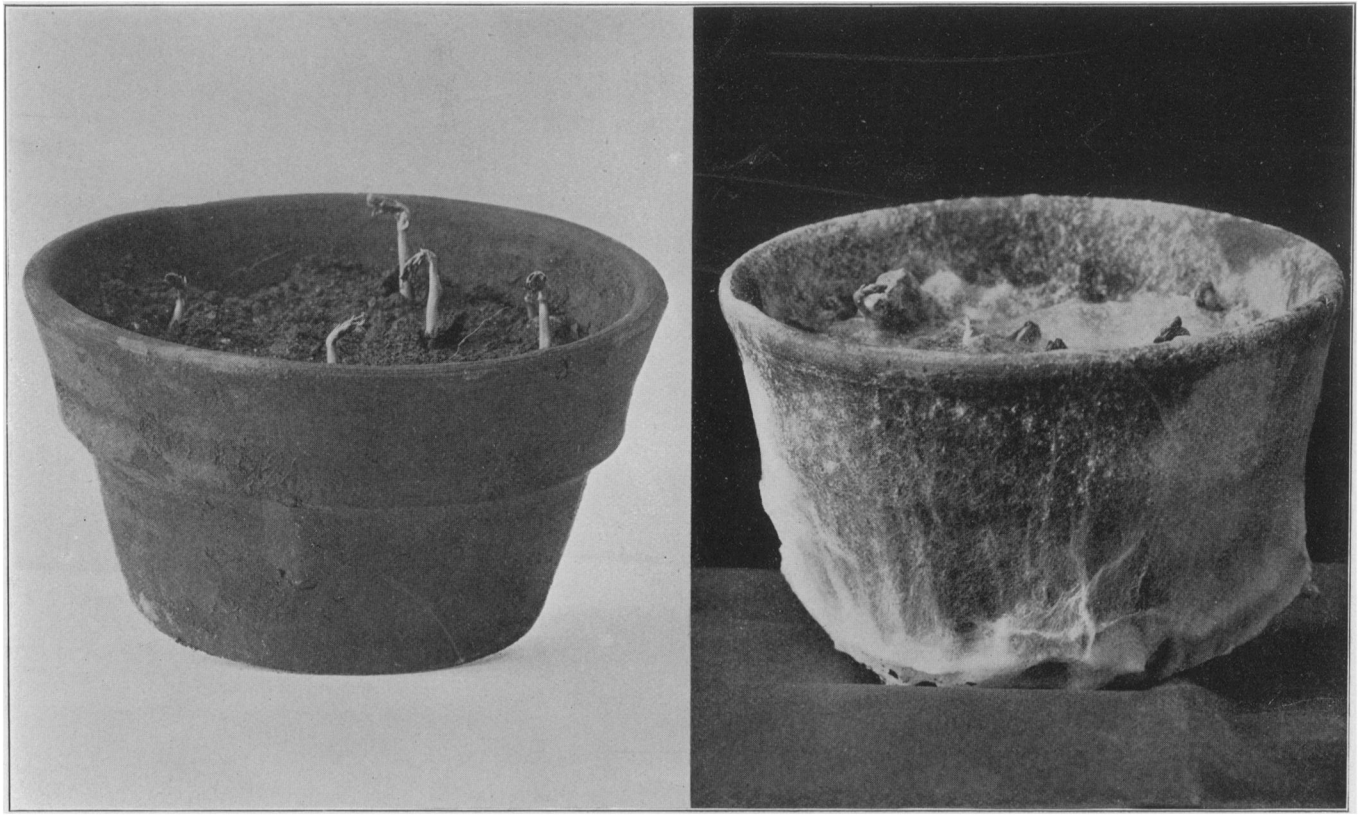
(WITH PLATES 9–12, CONTAINING TWENTY-ONE FIGURES)

To the collector it is a well-known fact that there are numerous species of fungi which are known only on burnt places. While some of these forms may occur under other conditions, such occurrence is so rare as to have attracted comparatively little attention. Many popular reasons have been offered by individuals in explanation of these facts, such as the elimination of competition in the destruction of the higher plants, the presence of carbon in the soil, and that these forms really occur in other habitats and escape detection, but none of these reasons is sufficient to explain the occurrence of at least one of the plants in question. That these fungi do not occur on burnt places simply because the competition of the higher plants has been eliminated is shown by the fact that they do not, as a rule, occur on bare soil which has not been burned over. My own observation has also shown that carbonaceous materials are not necessary to the life of some of the pyrophilous fungi, and we must look for other explanations of these interesting phenomena.

The genus *Pyronema* includes several species, which, as the name implies, commonly inhabit burnt places. The occurrence of the plants of this genus on burnt ground is sufficiently common

[MYCOLOGIA for May, 1909 (1: 83–130), was issued 4 June 1909.]



SOIL CULTURES OF PYRONEMA OMPHALODES

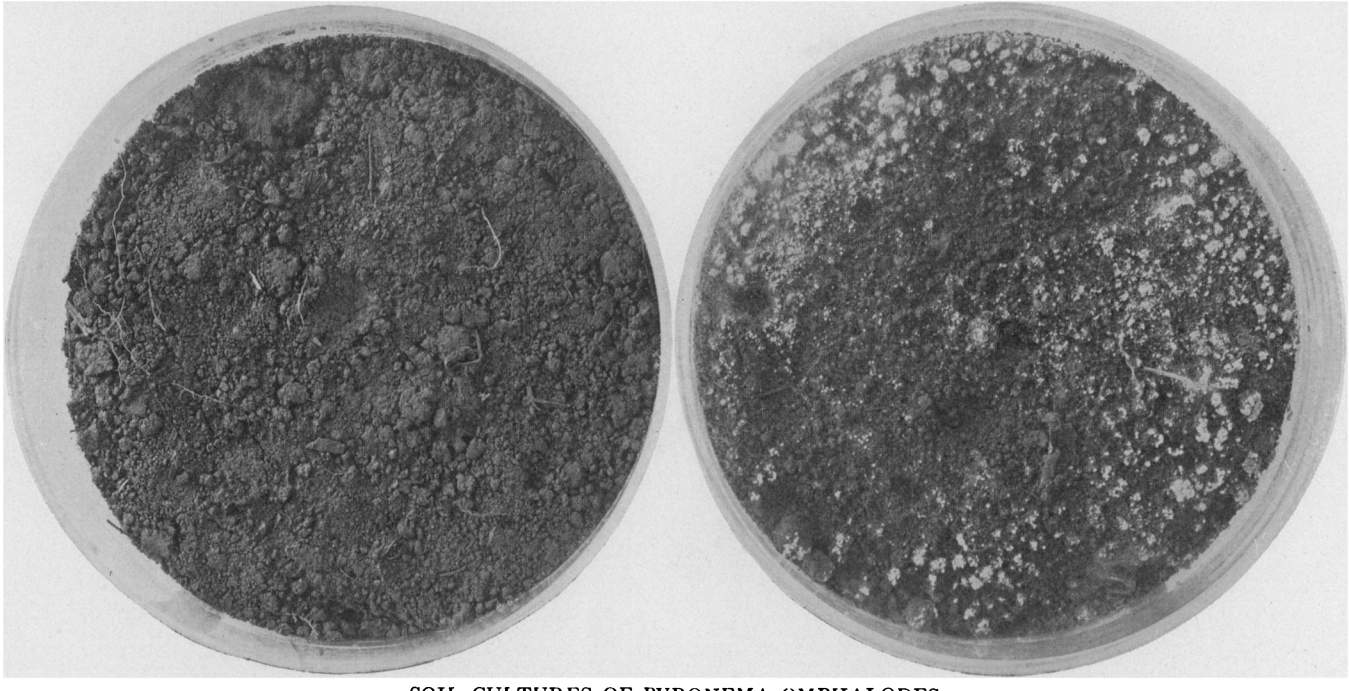
to have suggested the name of the genus, but no one has apparently considered the matter of sufficient importance to warrant investigation.* Since the most common species of the genus, *Pyronema omphalodes* (Bull.) Fuckel, is one of the few discomycetous fungi in which sexual reproduction has been demonstrated, numerous papers have been written on this phase of the subject, but in each case the matter of the occurrence of the species is dismissed with a simple statement of the fact. Nor, so far as known, has anyone taken the trouble to cultivate the species under artificial conditions either for the study of reproductive processes or in the attempt to gain information as to the reasons for its common occurrence on burnt ground.

The plants of this genus were first encountered by the writer in 1904, when the above-named species was found to be very common on burnt places near Iowa City, Iowa. Scarcely a burnt place could be visited in and about woods in wet weather on which this species was not found to be present and often in abundance, the plants appearing on charcoal and ashes and the burnt-over soil. While the individual plants are small, ranging from one to two millimeters in diameter, they commonly occur in dense confluent masses often covering a space of several inches, and by reason of their bright color they might, in spite of their small size, be counted among the more attractive forms of fungi.

The second occurrence of this species to attract the attention of the writer was during the fall of 1906 in the propagating houses of the New York Botanical Garden, where it was found to appear on soil sterilized with steam under a pressure of ten to fifteen pounds. Here the plants occurred as usual, forming rose-colored or salmon-colored sheets over the surface of the soil, the groups of plants being surrounded by a cobweb of mycelium. Under these conditions the plants seem to thrive for a time, but

* Since this paper went to press a synopsis of the article below has come to the attention of the writer showing that some of the conclusions drawn in the present paper have been previously arrived at. Although the present work was conducted without knowledge of this previous work and the line of experimentation is different, the conclusions, so far as the work has gone, are almost identical.

Kosaroff, P. Beitrag zur Biologie von *Pyronema confluens* Tul., gleichzeitig ein Beitrag zur Kenntniss der durch Sterilisation herbeigeführten Veränderungen des Bodens. Bot. Zeit. **66**: 23. 1908.



SOIL CULTURES OF PYRONEMA OMPHALODES

finally mature their fruit and disappear. The species was said to occur on soil sterilized in this manner almost without exception and had been noted for several years past by those carrying on experimental work here requiring the sterilization of soils; but as the fungus usually appeared before seeds had germinated and apparently did no harm, it did little more than to arouse a passing interest. The attention of the writer was at length called to this fungus and it was identified as *Pyronema omphalodes* (Bull.) Fuckel. The occurrence of a fungus commonly associated with burnt places on soil sterilized with steam was a fact of unusual interest, since it indicated that charcoal and carbonaceous materials are not necessary to the life of this fungus as was previously supposed.

In trying to explain these facts it at once became apparent that the high temperatures to which the substrata had been subjected had something to do with the appearance of these plants under the above conditions, but whether the high temperatures had some relation to the spores of the fungus itself in stimulating them to germination or to the substrata only in preparing it for the growth of the fungus was at that time a question.

During the summer of 1907 the species was again observed in North Dakota, where it occurred on bare soil by roadsides where there was no trace of charcoal, but in places which it is easy to suspect had been fire-swept or subjected to considerable temperatures by the heat of the sun and natural conditions of sterilization.

The last appearance of these plants and the one which has prompted the study of the problem which has been made the basis of the present paper was in agar which had been inoculated with the spores of other fungi in the laboratories of the New York Botanical Garden. The appearance of this fungus, uninvited, in three different cultures at the same time in a laboratory where to my knowledge none of the plants of the genus had been studied, even from dried material, for more than two years was sufficiently mysterious to arouse interest.

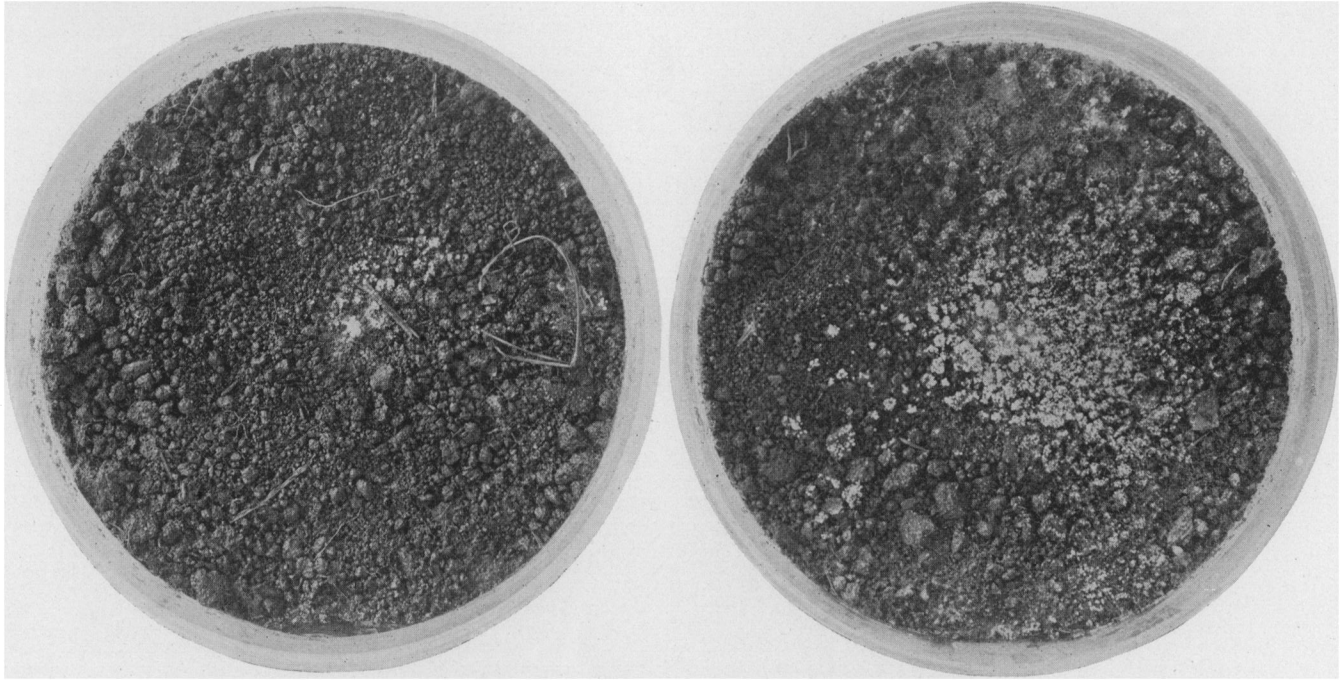
There were two possible explanations of the appearance of this fungus at this time; one that the cultures had become inoculated with the spores from the air and the other that the spores were present in the cultures and had withstood the process of steriliza-

tion. That the spores might not only be able to withstand the process of sterilization, but might even be stimulated to germination by high temperatures was suggested, since it is claimed that the spores of some of the coprophilous fungi must be subjected to the body temperature and other influences of the alimentary canal of animals in order to induce their germination.

In order to test the matter of the effect of heat on the spores of the fungus, mature ascospores were heated to various temperatures and later planted in hanging drop cultures. The heating was accomplished both with dry heat and by heating in a drop of water. In no case could the spores which had been heated to any considerable temperature be made to germinate. On the other hand, mature ascospores which had not been heated germinated readily in drop cultures, proving that if high temperatures have anything to do with the appearance of this fungus the effect is on the substratum only, the spores themselves being as sensitive to heat as are those of other species of fungi.

This species is not sufficiently common to expect that the air of the laboratory is saturated with the spores at all times, but from later experiments it is evident that these cultures were inoculated from the air. The fact that the fungus occurred in cultures in which the agar had been poured over filter paper previously heated to 110° C. for purposes of sterilization again raised the question of the relation existing between this fungus and the heating of the substratum. Cultures of agar were later tried, leaving out the filter paper, and the fungus was found to grow fully as well as in the preceding case. The luxuriant growth of this species on agar is evidence that high temperatures are not necessary in all cases to its growth. Soils sterilized with dry heat require a higher temperature to bring about favorable conditions for the growth of this fungus than are necessary for the sterilization of agar.

From our own observations and experiments there is little doubt that this fungus occurs on burnt places as a result of sterilization of such places by fire. However, it is probable that sterilization means much more than the simple elimination of competition by the destruction of bacteria and other fungi present in the soil. The nature of the changes brought about in soil by heating



SOIL CULTURES OF PYRONEMA OMPHALODES

to high temperatures is a question concerning which little is known and one which is of vital importance to the problem under consideration. While the heating of the soil destroys the fungi already present, there is every reason to believe that it prepares the way for the growth of those species which may be introduced subsequent to sterilization. The experimental work of the present paper has been based mainly on the one species, *Pyronema omphalodes* (Bull.) Fuckel, but the question of the effect of the heating of soils on the production of fungi is doubtless a large one and it is the intention of the writer to extend these investigations to other forms when fresh material can be secured for experimental work.

Some of the observations in support of the above conclusion are: in its occurrence on sterilized soil the fungus usually appears at a very early date and is mature before other forms of vegetation have had time to make any considerable growth; after maturing one crop of ascocarps the fungus gradually disappears, indicating that the most favorable time for its growth is immediately after sterilization; soil sterilized, moistened and allowed to stand for a week appears to be as unfavorable for the growth of *Pyronema* as soils which have never been sterilized, notwithstanding the fact that the soil is entirely free from other forms of vegetation so far as the eye can detect.

In its occurrence in nature on burnt ground, no notes have been made as to the relative time between the burning of the substratum and the appearance of the fungus. This would doubtless depend upon conditions of moisture. A place having been thoroughly sterilized would remain so until the return of moisture, when the *Pyronema* avails itself of the favorable conditions of sterilization and moisture and matures its crop of spores. So far as can be recalled, this species has been found on burnt places only when bare and apparently devoid of other forms of vegetation, indicating that it appears soon after burning or soon after the return of moisture to the burnt places.

In its occurrence on agar, the *Pyronema* grows rapidly, covering the surface of the agar in a three-inch petri dish in about four days. All of the cultures have been slightly contaminated with other fungi in the center of the dish, but fruit has not been

produced when the *Pyronema* has been planted in cultures already thoroughly contaminated with other fungi.

A fresh culture on agar in which the mycelium was radiating equally in all directions was contaminated by placing a drop of water rich in bacteria directly in front of the advancing mycelium. In a short time the water had evaporated, leaving only the contaminated spot. The mycelium continued to grow on both sides of the spot but refused to cross the infected area. Later, it gradually surrounded this area, which was apparently unfavorable to its growth.

In no case have I failed to produce an abundant crop of fruit in three to six days on soil sterilized under high steam pressure or with dry heat at a high temperature when such soils have been inoculated with the spores of the fungus. Indeed, such conditions are so favorable that it is difficult to prevent the fungus from invading such places even when not inoculated. On the other hand, in no case have I been able to produce more than a beginning of growth on unsterilized soil. Soils sterilized at low temperatures often produce a scant growth of ascocarps, which are, for the most part, devoid of the normal color.

The observation is made by Dr. R. A. Harper* that this plant also occurs on damp, well-rotted leaves where there has been no fire. I can account for this only on the ground that the leaves have been previously sun dried and subjected to natural conditions of sterilization, for in my experiments here every attempt to grow this fungus on unsterilized materials has failed. It is quite probable that other conditions of sterilization might give the same results as those produced by fire, but this point has not yet been demonstrated.

METHODS OF CULTIVATION FOR STUDY

The cultivation of fungi under artificial conditions is comparatively easy when we are able to meet the conditions in the laboratory under which they normally occur in nature. The apparent preference of this species for conditions of sterilization render it unusually favorable for cultivation under artificial conditions.

* Sexual Reproduction in *Pyronema confluens* and the Morphology of the Ascocarp. Ann. Bot. 14: 321. 1900.

The rapidity of growth, together with the fact that the sex organs in *Pyronema* are the largest known among the ascomycetes, should render the species of this genus of unusual interest to instructors who desire such material for study in the classroom, when the ease with which they may be artificially cultivated becomes known. The length of time during which the spores and mycelium will keep their vitality in the laboratory is a question which time alone will answer. When once the plant is started it can be cultivated generation after generation with perfect success, enabling the student to trace every step in the life-history of the plant from the germination of the spores to the production of the sex organs and, a few days later, the mature ascocarps.

The existence of sex organs in this plant has been known for many years, but it is only recently that Dr. R. A. Harper has demonstrated that these are actually functional. His study, however, was based on material collected under natural conditions, he having made no attempt to cultivate the species on nutrient media. The fact that this can be done would render the species as available for regular laboratory study as are the reproductive organs of some of the common algae.

If it is desired to study the reproductive organs from gross material, and agar is available, this is one of the best media to use, since the development of the plant can be studied in culture from day to day by placing it under the low power of a compound microscope. The surface of the agar is smooth and transparent, so that we may detect the earliest appearance of the forming fruit and these may be mounted on a slide in a drop of agar, thus eliminating grit and sand which might be present in material grown on soil. Much care must be taken to get the plants at a very early stage, for immediately after fertilization each cluster of sex organs is surrounded by the tissues of the developing ascocarp, which obscure the details of the reproductive organs.

Soil which has been heated to a high temperature is apparently more favorable for the production of the sex organs and ascocarps in large numbers than agar. In a pot of sterilized soil the fruit is produced on the pot as well as on the soil and can quite easily be removed for study. Since soil is always available and most nearly approaches the natural conditions for the growth of

the species, it is probably the most practical medium to be employed.

If plants are desired for sectioning, soft materials, such as broken leaves, may be placed on the soil and sterilized. In this case the fruit is formed in clusters on the leaves and soil. The pieces of leaves may then be removed, imbedded, and sectioned in the ordinary way, or the plants may be scraped off from the leaves and mounted and studied from the gross material.

SUMMARY

1. *Pyronema omphalodes,* which normally occurs on burnt places, can be successfully cultivated on nutrient media, producing sex organs on the fifth or sixth day and mature ascocarps in about ten days from the time of the planting of the spores.

2. This fungus will produce an abundance of fruit on soil or leaf-mold which has been sterilized by heating to high temperatures (110° C. or over), but refuses to produce fruit or any considerable mycelium on unsterilized soil or soil heated to low temperatures (less than 95° C.).

3. Sterilization by steam serves the same purpose as sterilization with dry heat, provided the soil is sterilized under sufficient pressure (5 lbs. or over). Soil sterilized under low pressure (2 lbs. or less) produces fruit only sparingly.

4. The time required to produce fruit on soil, as well as the abundance of the fruit itself, varies with the temperature to which the substratum has been subjected. Soil sterilized at 95° C. has produced no fruit; soil sterilized at 110° C. produces a fair quantity of fruit; while soil sterilized at 135°–145° C. produces fruit in abundance. The length of time of the application of the heat also has some influence.

5. Sterilization of soil by heat apparently brings about some change in the soil other than the simple elimination of competition in the destruction of bacteria and other fungi, which changes appear to be of vital importance in the cultivation of fungi which normally grow on burnt soil.

PYRONEMA OMPHALODES

EXPLANATION OF PLATES

PLATE IX

Two pots of soil, the left unsterilized, the right sterilized with dry heat at 140° C. for 15 hrs. Both were planted with seeds of pea, the sterilized pot soon becoming thoroughly infected with *Pyronema omphalodes* (Bull.) Fuckel, the unsterilized pot remaining uninfected. × ⅔.

PLATE X

Soil cultures, the left unsterilized, the right sterilized with steam under a pressure of 5 lbs. for 1–2 hrs. Both were innoculated with the spores of *Pyronema omphalodes* at a point near the center of the culture. The unsterilized culture produced no fruit and a very scant growth of mycelium surrounding the point of inoculation. The sterilized culture produced an abundant growth of mycelium and abundant fruit. × ½.

PLATE XI

Soil cultures, the left sterilized with dry heat at 110° C. for 1 hr., the right sterilized at 145° C. for 1 hr., the latter producing mycelium and fruit in much greater abundance than the former. × ½.

PLATE XII

1–2. Germination of spores of *Pyronema omphalodes* in hanging culture after 20 hrs.
3. Portion of mycelium drawn from culture grown on agar.
4. Portion of a cluster of oogonia at an early stage.
5. Cluster of oogonia drawn from material grown on agar.
6. Cluster of oogonia partially teased out.
7–13. Figures of oogonia and antherida drawn from material grown on agar.
14–15. End views of oogonia and antheridia drawn from culture material.

All the figures on this plate were sketched with the aid of a camera lucida, and are magnified 500 diameters.

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